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L.M.B.C. MEMOIRS

XXVI.

BOTRYLLUS

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The Committee desire to intimate that no copies of these Memoirs will be presented or exchanged, as the prices have been fixed on such a scale that most of the copies will have to be sold to meet the cost of production.

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Memoir I. Ascidia—published in October, 1899, 60 pp. and five plates, price 2s.
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,, III. Echinus—published in February, 1900, 36 pp. and five plates, price 2s.
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,, XXVI. Botryllus—published in October, 1924, 52 pp. and six plates, price 4s. 6d.

Liverpool Marine Biology Committee.

L.M.B.C. MEMOIRS

ON TYPICAL BRITISH MARINE PLANTS & ANIMALS

EDITED BY W. A. HERDMAN, C.B.E., F.R.S.,
AND JAMES JOHNSTONE, D.Sc.

XXVI.

BOTRYLLUS

BY

E. CATHERINE HERDMAN, M.Sc.

(With 6 Plates)

THE UNIVERSITY PRESS OF LIVERPOOL LTD.
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EDITORS' PREFACE.

The Liverpool Marine Biology Committee was constituted in 1885, with the object of investigating the Fauna and Flora of the Irish Sea.

The dredging, trawling, and other collecting expeditions organised by the Committee have been carried on intermittently since that time, and a considerable amount of material, both published and unpublished, has been accumulated. Thirty-six Annual Reports of the Committee and five volumes dealing with the "Fauna and Flora" have been issued. At an early stage of the investigations it became evident that a Biological Station or Laboratory on the seashore nearer the usual collecting grounds than Liverpool would be a material assistance in the work. Consequently the Committee, in 1887, established the Puffin Island Biological Station on the North Coast of Anglesey, and later on, in 1892, moved to the more commodious and accessible station at Port Erin in the centre of the rich collecting grounds at the south end of the Isle of Man. A larger Biological Station and Fish Hatchery, on a more convenient site at Port Erin, has since been erected, and was opened for work in July, 1902. In 1920 the Committee ceased to exist and the management of the station was transferred to the Oceanography Department of the University at Liverpool. The name "L.M.B.C. Memoirs" is, however, still retained.

In these thirty-six years' experience of a Biological Station, where University students and amateurs form a large proportion of the workers, the want has been frequently felt of a series of detailed descriptions of the structure of certain common typical animals and plants, chosen as representatives of their groups, and dealt with by specialists. The same want has probably been felt in other similar institutions and in many University laboratories.

The objects of the Committee and of the workers at the Biological Station were at first chiefly faunistic and speciographic. The work must necessarily be so when opening up a new district. Some of the workers have published papers on morphological points, or on embryology and observations on life-histories and habits; but the majority of the papers in the volumes on the "Fauna and Flora of Liverpool Bay" have been, as was intended from the first, occupied with the names and characteristics and distribution of the many different kinds of marine plants and animals in our district. And this faunistic work will still go on. It is far from finished, and the Committee hope in the future to add still further to the records of the Fauna and Flora. But the papers in the present series, started in 1899, are quite distinct from these previous publications in name, in treatment, and in purpose. They are called "L.M.B.C. Memoirs," each treats of one type, and they are issued separately as they are ready, and will be obtainable Memoir by Memoir as they appear. It is hoped that such a series of special studies, written by those who are thoroughly familiar with the forms of which they treat, will be found of value by students of Biology in laboratories and in Marine Stations, and will be welcomed by many others working privately at Marine Natural History.

The forms selected are, as far as possible, common L.M.B.C. (Irish Sea) animals and plants of which no adequate account already exists in the text-books. Probably most of the specialists who have taken part in the L.M.B.C. work in the past will prepare accounts of one or more representatives of their groups. The following is a list of the Memoirs now published:—

Memoir I. Ascidia, W. A. Herdman, 60 pp., 5 Pls., 2s.
,, II. Cardium, J. Johnstone, 92 pp., 7 Pls., 2s. 6d.
,, III. Echinus, H. C. Chadwick, 36 pp., 5 Pls., 2s.
,, IV. Codium, R. J. H. Gibson and Helen Auld, 26 pp., 3 Pls., 1s. 6d.
,, V. Alcyonium, S. J. Hickson, 30 pp., 3 Pls., 1s. 6d.
,, VI. Lepeophtheirus and Lernæa, Andrew Scott, 62 pp., 5 Pls., 2s.
,, VII. Lineus, R. C. Punnett, 45 pp., 4 Pls., 2s.
,, VIII. Plaice, F. J. Cole and J. Johnstone, 260 pp., 11 Pls., 7s.
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,, XI. Arenicola, J. H. Ashworth, 126 pp., 8 Pls., 4s. 6d.
,, XII. Gammarus, M. Cussans, 55 pp., 4 Pls., 2s.
,, XIII. Anurida, A. D. Imms, 107 pp., 8 Pls., 4s.
,, XIV. Ligia, C. G. Hewitt, 45 pp., 4 Pls., 2s.
,, XV. Antedon, H. C. Chadwick, 55 pp., 7 Pls., 2s. 6d.
,, XVI. Cancer, J. Pearson, 217 pp., 13 Pls., 6s. 6d.
,, XVII. Pecten, W. J. Dakin, 144 pp., 9 Pls., 4s. 6d.
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Memoir XXII. ECHINODERM LARVÆ, H. C. Chadwick, 40 pp., 9 Pls., 2s. 6d.
„ XXIII. TUBIFEX, G. C. Dixon, 108 pp., 7 Pls., 3s. 6d.
„ XXIV. APLYSIA, Nellie B. Eales, 92 pp., 7 Pls., 4s. 6d.
„ XXV. ASTERIAS, H. C. Chadwick, 72 pp., 9 Pls., 4s. 6d.
„ XXVI. BOTRYLLUS, E. Catherine Herdman.

It may be well to explain briefly why *Botryllus* has been chosen as the subject of this memoir.

In the first of this series,* W. A. Herdman stated that the Compound Ascidians, of which *Botryllus* is here taken as an example, are probably an artificial assemblage consisting of several groups derived from Simple Ascidians which have independently acquired the colonial mode of growth—" colonies in which the ascidiozooids are so intimately united that they possess a common test or investing mass." It had previously been pointed out in the same author's Report on the Challenger Tunicata (Part II, 1886, p. 387, etc.), and elsewhere, that the Compound Ascidians must be regarded as a polyphyletic group, and that the family Botryllidae is probably derived from one group (the Styelidae) of the Simple Ascidians, while other families of Compound Ascidians are more nearly related to such Simple Ascidians as *Clavelina* and *Ascidia*.

This view as to the relationships of these families has been adopted by most subsequent writers, but has resulted, in some recent classifications, in the Botryllidae being separated from other Compound Ascidians and united to the Styelidae—a position which may indicate their past origin but does not represent their present habit and structure. Whatever their history has been in the past, the Botryllidae are now in structure and appearance Compound Ascidians formed of colonies of ascidiozooids produced by gemmation and imbedded in a common test.

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* L.M.B.C. Memoir I, *Ascidia*, 1899.

Botryllus was, therefore, considered to be available as a Compound Ascidian, and has been chosen as the representative of the group for this series of memoirs because of certain advantages which render it most suitable for the purposes of University Laboratories and Biological Stations: (1) It is abundant and widely distributed in the littoral zone of most European and North American coasts, and can usually be obtained at any season; (2) it is of fairly large size, and consequently, the ascidiozooids can be extracted from the common test and dissected under the microscope without much difficulty; (3) it shows very beautifully one of the methods of gemmation known in Compound Ascidians; and usually many stages in the development of buds may be found in a single colony.

The relative positions of *Botryllus* and *Ascidia*, etc., can be seen from the following abbreviated classification of Tunicata:—

I. LARVACEA—minute, tailed, larva-like, free-swimming (*Oikopleura*).

II. THALIACEA—tail-less, free-swimming, highly modified (*Salpa, Doliolum*).

III. ASCIDIACEA—sessile Simple and Compound Ascidians.
  1. Simple Ascidians (*Ascidia*, etc.).
  2. Compound Ascidians (*Botryllus*, etc.).
  3. The Compound, free-swimming, *Pyrosoma*.

In the memoir on *Ascidia*, a Simple Ascidian, it was promised that a later memoir in the series would deal with *Botrylloides* as a type of the Compound Ascidians, and the announcement has been continued in the list of forthcoming memoirs since. Of late years, however, *Botryllus* has been substituted for *Botrylloides* in the list, a slight change which presented no disadvantages and was justified by the experience that *Botryllus* was more usually examined in the University Laboratories, more frequently seen in Museums and more abundantly collected on the seashore and brought into Biological Stations. Its brilliantly coloured fleshy crusts under stones and on sea-weeds, its curious circular systems of radiating ascidiozooids, and the elaborate series of vascular tubes and highly pigmented terminal knobs in the gelatinous test excite the interest of all young Naturalists who find it on their collecting expeditions or see it alive in aquaria.

W. A. HERDMAN.
JAMES JOHNSTONE.

OCEANOGRAPHY DEPARTMENT,
UNIVERSITY OF LIVERPOOL,
July, 1924.

AUTHOR'S PREFACE.

THIS memoir should be used in conjunction with No. 1 of the series—" Ascidia," by W. A. Herdman. The two animals, *Ascidia* and *Botryllus*, although so different in appearance, are essentially similar in structure and development, and many points—notably the embryology and the classification of the whole group—are dealt with very much more fully in the earlier memoir. Thus the student will notice those differences which do occur and realise how they are connected with the different life-history.

I should like to take this opportunity of expressing a deep sense of indebtedness to my father, Sir William Herdman, for his constant helpful criticism and advice throughout my work. I also thank Professor F. J. Cole for a large collection of slides of Compound Ascidians prepared by him, Mr. J. R. Bruce, of the Port Erin Biological Station, who collected and preserved material for me at a time when I was unable to be at Port Erin myself, and Mr. J. Duggan, of the Hartley Botanical Laboratory, who took the photomicrographs showing the structure of the stomach wall. I am grateful to Professor James Johnstone for his advice in preparing the memoir for publication, and to Professor W. J. Dakin, in whose department the work was carried out.

I have made free use of the published results of previous workers and have decided not to burden the text with references. For the use of those students who wish to consult the original authorities I have appended a list of some of the principal papers dealing with the Botryllidae (p. 35). An excellent bibliography of the Tunicata as a whole is that of J. Hopkinson (Ray Society Memoirs, 1912).

E.C.H.

L.M.B.C. MEMOIRS

No. XXVI. BOTRYLLUS

BY

E. CATHERINE HERDMAN, M.Sc.

IN the year 1755, Albert Schlosser dredged up, from shallow water in Falmouth Harbour, a stem of *Fucus* covered by a hard but fleshy substance, in which there were numerous brightly coloured star-like structures. " I put it," he says, " . . . . into a bucket of sea-water, expecting every moment that the polypes, which I thought to lodge in those little stars, would extend and show themselves like those of the alcyonium, ... commonly called dead-man's hand ; but after more than half an hour's fix'd attention, the vessel lying very quiet all the time, I did not perceive the least appearance of any polypes ; upon which I brought them to shore . . . . , and then, by means of my microscope, I discovered every one of those stars to be a true animal, and much more beautiful than any polype, but quite of a different structure."

By showing these specimens at the Royal Society of London, Schlosser drew the attention of biologists to this hitherto almost unknown animal, whose structure and systematic position puzzled them for many years. It was Savigny, half a century later, who finally showed that it was a colonial animal related to the Simple Ascidians, and who formed the genus *Botryllus*, corresponding to the whole family of the Botryllidae, Giard, as it is known to-day.

OCCURRENCE AND EXTERNAL APPEARANCE.

Botryllus is one of the most widespread of shallow water marine organisms. It occurs on all rocky shores around the coasts of Europe, North America and the Red Sea, and possibly further investigation will show it to be almost cosmopolitan. It ranges from a little above the level of low water down to a depth of about 15 or 20 fathoms, and is found encrusting rocks and boulders, especially on the sheltered or lower surface; it also spreads over the stems of *Fucus*, *Laminaria* and *Zostera* and often forms a thick covering on floating buoys, lobster-pots, mooring ropes, etc.*

The brilliant and varied colouring of the flower-like groups of ascidiozooids places *Botryllus* amongst the most decorative of the many beautiful plants and animals which inhabit a rocky shore, where it forms a suitable background for the brightly coloured and graceful Nudibranchs, worms and Crustacea, which live along with and often devour it.

When the living colony, or **corm,** of *Botryllus* is examined with a lens, it becomes evident that each of the flower-like **systems** consists of several animals measuring about 2 mms. in length, arranged radially (see fig. 1). Each of these little animals is known as an **ascidiozooid,** and corresponds in structure to one of the much larger Simple Ascidians, some of which reach a size of nearly a foot in length. In the centre of the system a circular or elliptical opening is visible and this is the common cloaca, into which the atrial or exhalant aperture of each ascidiozooid empties. The branchial or inhalant aperture is at the anterior end of the body, which is directed radially outwards, and so is far removed from the common cloaca. Thus all the ascidiozooids in one system are intimately connected by their atrial apertures, and a closer examination reveals a further connection. Throughout the tough gelatinous test, in which the whole system is embedded, run numerous fine branching and anastomosing blood vessels with swollen terminal knobs, where they approach the surface. Each ascidiozooid gives off two main vessels from its ventral surface and all these, together with their many branches, form this common external circulatory system in the test.

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* Pizon has shown that *Botrylloides* is unable to carry on its normal activities when deprived of light. This accounts for the bathymetric distribution of the Botryllidae in shallow water only. There is only one record of a *Botrylloides* dredged from deep water (530 fathoms) by the Lightning Expedition—and as this specimen was apparently not attached to the bottom it may possibly have drifted from shallower water.

LIFE-HISTORY.

The corm often covers an area of many square inches and contains hundreds of systems, yet all these may be the product of a single fertilised ovum, for *Botryllus* possesses, in a high degree, the power of asexual reproduction or budding. During the early summer months eggs are formed. They are fertilised and begin their development within the body of the parent, and are then liberated as tadpole-shaped larvae. These swim for about two days, then attach themselves to some rock or seaweed and the tail is absorbed.* So far this is the typical development of any Ascidian, but, even during the larval stage of *Botryllus*, a change has begun to take place. On each side, the outer wall of the peribranchial cavity has become thickened and has formed a slight evagination. On the left side, no further change takes place, but when the larva, or **oozooid,** has become fixed, the evagination on the right side grows out into a double-walled sac joined to the oozooid by a narrow stalk. This double-walled sac is the first bud, or **blastozooid** and it rapidly differentiates so as to resemble, in its essential features, the oozooid, which now begins to degenerate. The blastozooid, in turn, forms two lateral bulges and both of these develop. Thus the second generation of blastozooids consists of two ascidiozooids. These move apart until they lie opposite one another, connected only by their atrial apertures, and each of these again forms two buds. These four, still connected, form the third generation of blastozooids. This bilateral budding is the rule among the blastozooids, so that theoretically each system should consist of a number of ascidiozooids represented by some power of two (see Text-fig. 1). As a matter of fact, abortions are frequent, especially as the system becomes more crowded. When there is no longer room for all the ascidiozooids around the original cloaca, one or more may be pushed out into the surrounding test (see fig. 1). Here they continue to form lateral buds and so found new systems. In this way the colony may go on increasing indefinitely, so long as conditions are favourable.

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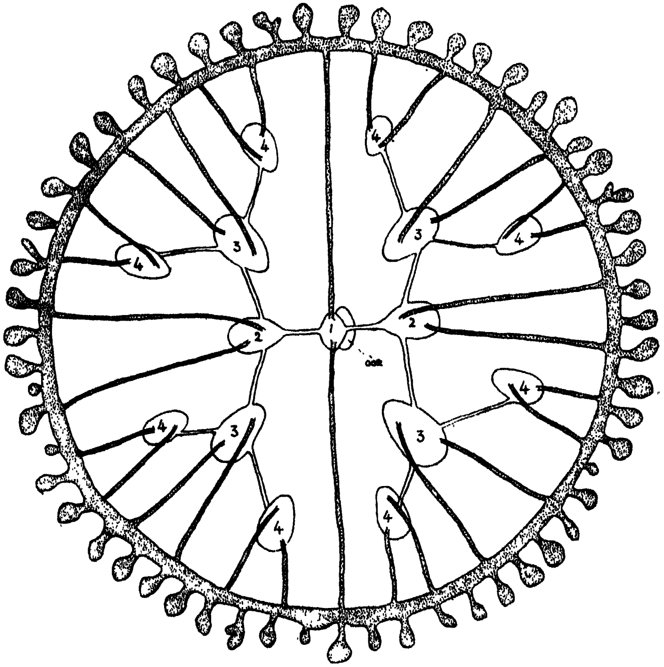
* Cf. the metamorphosis of *Ascidia*. L.M.B.C. Memoirs, No. 1, by W. A. Herdman.

All large colonies, however, are not necessarily formed from a single oozooid. Several larvae may attach themselves close together and form small colonies, which will ultimately fuse indistinguishably with one another; the vessels of the test anastomose freely and, possibly, within a single system, ascidiozooids may be found descended from separate oozooids.

This method of budding has been described as parietal, and is usually considered to be the only method of asexual reproduction in *Botryllus*. It is quite possible, however, that occasionally certain swollen knobs on the blood vessels of the test may give rise to buds. Cases have been recorded where this appears to be taking place and a similar method of budding is known in other Ascidians, but it is certainly not usual in *Botryllus* and there is no definite proof that it has ever occurred.



TEXT-FIG. 1. Diagram of a young colony of *Botryllus*, illustrating the method of budding and the arrangement of the blood vessels. The oozooid and four generations of blastozooids are represented. The blood vessels are stippled while the peduncles of the buds are left blank. [Partly after Delage and Hérouard im. Pizon.]

ooz. = oozooid. 1, 2, 3, 4 = first, second, third and fourth generations of blastozooids.

HIBERNATION.

Most Compound Ascidians undergo a period of hibernation during the colder months of the year. During this period, the vital processes are retarded and, in some cases, the ascidiozooids die down, leaving buds, which remain undeveloped until the following spring.

In *Botryllus* the winter changes are slight, consisting merely in a slowing down of all activities. Budding is very much reduced and the gonads remain undeveloped.

INDIVIDUALITY.

It is impossible to consider the life-history of *Botryllus* without discussing the question: "What is the individual?" T. H. Huxley defined an individual as "the total product of a single impregnated ovum," but the phenomena of parthenogenesis and identical twinning have shown that this definition is not applicable to all cases. In *Botryllus*, it would mean that the whole corm may be one individual or several such, indistinguishably fused, which would certainly be an unsatisfactory state of affairs. Another definition of the individual is that it has the power of living and continuing to live alone. According to this, each ascidiozooid is an individual, for the first bud always lives an independent life, and any bud of a later generation can live alone, if the other ascidiozooids of the system are destroyed without damaging it. On the other hand, the ascidiozooids *are* connected and a certain degree of co-operation exists between them. They are parasitic upon one another to the extent that the substance of degenerating individuals is absorbed into the common test vessels and distributed to the remaining ascidiozooids. Bergson and his school, reviving and modifying the older views of Perrier, divide living organisms into several orders of individuality, but this is, at any rate to some extent, begging the question of *what* an individual is.

Hickson considers that the word "individual" should be used to express "a discontinuity of an organism in time and space." Then the whole corm of *Botryllus* with its common test and common colonial vascular system is one individual,* but, at any moment, it may be broken up to form several individuals, or, on the other hand, two such individuals may, by concrescence, become one individual. This conception certainly avoids many of the absurdities of the older definitions. Perhaps its only disadvantage is that it gives to the word " individual " in biological usage a special meaning very different from that of ordinary speech. The dictionary definition of the term is " not divisible without loss of identity "—a phrase which is clearly inapplicable to the corm of *Botryllus.* The only part of the colony which is not divisible without loss of identity is the ascidiozooid, and, since the blastozooid is practically indistinguishable in structure from the oozooid, which is undoubtedly an individual, and since it can, under suitable circumstances, live independently, it would seem most reasonable, if the matter *must* be decided, to consider all the ascidiozooids as individuals. Probably, however, there is no other class of animals in which the individuals are more intimately connected than in the Compound Ascidians, and indeed it may be said that, in the evolution of their complex life-history, they have to some extent lost their individuality—a word which is, therefore, best avoided altogether in connection with *Botryllus* and the class for which it stands.

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* Bancroft, also, comes to this conclusion. He, however, distinguishes between a "biological individual" (Huxley's definition) and a "morphological and physiological individual, an individual localised in a single mass and functioning as a co-ordinated whole."

TEST (see figs, 16 and 17).

The test is a structure characteristic of all the Tunicata, but while, in the Simple Ascidians, such as *Ascidia,* it forms a tough leathery or gelatinous " tunic " investing the body of the animal and acting as an organ of attachment, in the Compound Ascidians it is enormously developed and appears as a mass of firm jelly, in which the tiny ascidiozooids lie embedded. In *Botryllus*, the test is of the encrusting type and often covers wide expanses, although it rarely attains a thickness of more than a few millimetres. It is closely applied to all the irregularities of the substratum, to which it adheres so firmly that, when the corm is removed from soft or brittle rocks, such as the Manx Slate at Port Erin, it is usual to find pieces of the rock itself coming away with the animal.

Histology. Although the test appears to be a clear, structureless jelly, microscopical examination shows fine interlacing fibrils running all through it, which closely resemble the delicate unstriped muscle fibres of the mantle. There are also numerous cells embedded in the test and these are known to have migrated from the body of the ascidiozooid. Both these test cells and the ectoderm cells over the whole surface of the ascidiozooid and especially those of the terminal knobs of the test vessels, secrete the non-cellular gelatinous matrix of the test containing " tunicine," a substance allied to the cellulose of plant tissues.

Growth. Throughout the life of the colony the test is continually added to by secretion of tunicine from the cells of the ectoderm and by mesoderm cells, which have migrated into the test. At the same time, the surface is continually being worn away, and shreds and films of ragged test are cast off. Thus, although in a healthy and active colony the test increases in superficial area, the thickness remains more or less constant.

Pizon has shown that in a colony of *Botrylloides* placed in the dark, development is arrested—that is, the ascidiozoöids degenerate, the test vessels being filled with cells derived from them, but the circulation still continues, showing that the colony is not dead. Under these circumstances, the test becomes thinner and thinner for loss still takes place from the surface and this is no longer replaced by secretion. It is evident then that the test depends, for its existence, on the continued activity of the organism as a whole and, directly or indirectly, on the presence of light.

ANATOMY OF THE ASCIDIOZOOID.

GENERAL PLAN (see Plate II)

The adult ascidiozooid lies embedded in the test in an almost horizontal position ; the antero-dorsal region is towards the surface of the test, while the postero-ventral region is nearest to the substratum. The branchial aperture is circular and can be closed by a double sphincter muscle when conditions are unfavourable for the intake of water. Within these sphincters is a ring of eight tentacles, four large alternating with four smaller ones, and within these again the water passes into the branchial sac, which occupies the greater part of the body. The branchial sac is roughly cylindrical in shape and its walls are perforated by numerous ciliated gill slits, or stigmata, through which the water can pass into the peribranchial or atrial cavity. This cavity is continuous except along the mid-ventral line, where the branchial sac is attached to the body-wall. In the postero-dorsal region, the atrial cavity opens by the atrial aperture into the common cloacal cavity, and so to the exterior. At the posterior end of the branchial sac is the entrance to the oesophagus, and the alimentary canal forms a loop on the left side of the branchial sac, the anus opening into the atrial cavity. The hermaphrodite gonads are paired and lie one on either side of the branchial sac.

BODY-WALL.

The body-wall, or mantle, of *Botryllus* is similar to that of all typical Ascidians. It consists of an outer layer of squamous epithelium, the ectoderm, which is in contact with, and, to a great extent, secretes the test. This ectoderm is continuous over the entire surface of the body, except at the branchial and atrial openings, where it is turned in for a short distance accompanied by a thin film of test (see fig. 2). Within the ectoderm lies a layer of mesoderm, consisting of connective tissue penetrated by numerous blood sinuses and by muscle and nerve fibres. So extensive are these blood sinuses in many parts of the mantle, that it has the appearance of a great blood space crossed by fine strands of mesoderm cells. The connective tissue cells of the mesoderm, many of which also migrate into the test and help to secrete its matrix, are very variable in form. They may become fusiform, stellate, or amoeboid, while others are enlarged and vacuolated and are known as " bladder cells." The muscle fibres are poorly developed, except around the branchial and atrial apertures, where they form sphincter muscles.

The lining of the atrial cavity, which forms the inner limit of the mantle over most of its extent, has sometimes been called the " third tunic," the test and the mantle proper being the first and second tunics—hence the name Tunicata.

BODY CAVITIES.*

The various spaces in the body of the adult ascidiozooid are the same as those found in the Simple Ascidians, but the origin of these cavities in the germ layers of both oozooid and blastozooid has evoked much discussion among the various investigators. As these facts of development are of interest, not only in connection with *Botryllus*, but also in their bearing on the fundamental conception of the germ layers, it is necessary to give here a brief summary of the case as at present understood.

In the **oozooid** :—

1. The alimentary canal, including the branchial sac and the pyloric caecum, represent the embryonic archenteron. They are lined by *endoderm.*

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* See L.M.B.C. Memoirs, No. 1, *Ascidia,* by W. A. Herdman.

The cavity of the refringent organ is variously interpreted as an outgrowth from the gut (*endodermic*) and as a part of the blood system (*blastocoelic*).

2. The peribranchial or atrial cavity is formed, at an early stage, as two lateral outgrowths from the undifferentiated alimentary canal (mesenteron), and is, therefore, lined by *endoderm*. Morphologically, it is in close connection with the exterior and it must be remembered that, in the Simple Ascidians, the cavity is formed as two symmetrical *ectodermal* invaginations, which later join dorsally to form the single atrium.

The perivisceral cavity results from two backward outgrowths from the atrial cavity.

Simultaneously with the formation of the peribranchial sacs, two median outgrowths appear, one from the dorsal and one from the ventral wall of the gut (mesenteron). The former gives rise to the neural gland and the latter to the pericardium. Both these cavities also are therefore endodermal in origin.

3. The cavity of the gonads is formed as a split in the mesoderm and is therefore *schizocoelic*.

4. The blood spaces of the body, including the heart, are derived from the segmentation cavity of the embryo, i.e. they are *blastocoelic*.

In the **blastozooid:** —

The inner vesicle of the bud is formed from the peribranchial cavity of the oozooid, i.e. it is bounded by *endoderm*. This vesicle gives rise to the branchial sac, gut, atrium, perivisceral space, pericardium and the cavity of the neural gland and its duct (cf. 1 and 2 above).

The cavity of the gonads is formed, as in the oozooid, as a split in the *mesoderm*.

The blood space of the bud is continuous with that of the parent.

It is impossible to believe that the various organs in *Botryllus* are not homologous with the corresponding parts in the Simple Ascidians. It must be supposed, then, that the germ layer theory is not infallible or, at any rate, that the endoderm may retain enough of its embryonic character to be able to take over the formation of the atrium, an organ produced in other Ascidians by the ectoderm.*

BRANCHIAL SAC.

The branchial sac is really the anterior part of the alimentary canal, and corresponds to the pharynx in other higher animals. The presence of gill slits in its wall is typical of the whole group Chordata, but in none is this modification as an organ of respiration and food capture carried so far as in the Tunicata. In the adult *Botryllus,* there are usually between 300 and 500 stigmata or secondary gill slits, formed by the division and multiplication, in the oozooid, of the primary larval gill slits. These are arranged on each side in from six to eleven transverse rows, and each row usually consists of from twenty-one to twenty-five stigmata. Occasionally, however, a few large gill slits are found taking the place of a number of smaller ones. This is unusual and may be due to fusion of several neighbouring stigmata, to imperfect division during development, or to abnormal healing of some injury.

The gill slits are separated from one another by transverse and longitudinal bars (see fig. 8), in the thickness of

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* The distinction does not seem so great when it is considered that in both cases the cavity is really formed by a double process—outgrowths from the alimentary canal meeting with ectodermal invaginations from the exterior. In the Simple Ascidians the endodermal element is so small as to be negligible, forming as it does no more than a part of the primary gill slits, while in the Botryllidae it bounds the whole cavity of the atrium and limits the ectoderm to the lining of the atrial aperture. The difference may be regarded then as one merely of degree.

which run channels in which the blood circulates. The transverse gill bars are thicker and more regular than the longitudinal bars. Besides these, there are, on each side of the branchial sac, three internal longitudinal bars (see fig. 2), which are thicker and stronger than the external longitudinal bars, and which are continuous from end to end of the branchial sac. They are connected with the transverse bars and are so placed that in each row there are seven or eight stigmata between the mid-dorsal line and the first internal longitudinal bar, four or five between each internal longitudinal bar and the next, and six or seven between the third internal longitudinal bar and the mid-ventral line (see fig. 2), giving the following formula:—
D.L., 7, I, 4, II, 4, III, 6, En.

The walls of the stigmata are ciliated and so a current of water is maintained, sweeping in through the branchial aperture and out through the gill slits. By this means, the blood circulating in the walls of the branchial sac may be aerated, and a supply of food material brought within reach of the glandular and ciliated apparatus of the endostyle, peripharyngeal bands and dorsal lamina, which all co-operate to sweep these minute particles into the oesophagus.

The branchial sac is held in position within the peribranchial cavity by vascular connectives or trabeculae running from the mantle to the branchial sac. These usually open into the transverse bars between the internal longitudinal bars.

TENTACLES.

Situated a short distance within the branchial aperture is a ring of eight simple finger-like tentacles, four large ones alternating with four small (see figs. 2, 3, and 5). In development these do not all appear simultaneously. The two medio-lateral which lie perpendicular to the long axis of the body appear first ; the next to develop is the mid-ventral one, then the mid-dorsal one followed very closely by the smaller latero-ventral tentacles. The two latero-dorsals are the last to appear. Thus at various stages in the growth of the buds, the tentacles appear as two, three, four or six in number, but probably the complete number is always eight.

It has been suggested that these tentacles are testing organs and that, if the inflowing water is impure, a stimulus will be transmitted through them, which will cause the branchial aperture to close, or the whole body to contract violently, ejecting the water through both openings. They probably also act as a sieve by preventing large particles from entering the widely opened branchial aperture. In life, the tentacles are extended across this opening, so as almost to touch in the centre, the four shorter ones filling in the spaces between the bases of the four larger (see fig. 5).

ENDOSTYLE.

Along the mid-ventral line of the branchial sac runs a deep groove, the endostyle, in which six longitudinal bands of columnar glandular cells alternate with five bands of closely packed ciliated cells (see fig. 12). The cilia of the mid-ventral band are very long. Those of the lateral bands are short. The lips of the endostyle are covered by an epithelium of cubical ciliated cells. This ciliated, glandular groove, which is a very constant and characteristic feature of all Tunicates, extends almost to the posterior end of the branchial sac. Here it narrows and the ciliated lips close in to form a simple ciliated ridge known as the posterior fold (see fig. 18), which passes back to merge with the right lip of the oesophageal opening.

It was first shown by W. Müller that the endostyle of the Tunicata is homologous with the hypopharyngeal groove of Amphioxus and the thyroid gland of the Vertebrata.

PERIPHARYNGEAL BAND.

In the majority of the Tunicata a pair of ciliated ridges pass round the anterior end of the pharynx and are known as the peripharyngeal bands. In *Botryllus* the posterior band is well developed. It is a ciliated ridge of epithelium situated in the prebranchial zone of the pharynx. In the mid-ventral line it is continuous with the ciliated lips of the endostyle (see fig. 9), while in the mid-dorsal line its two ends are deflected posteriorly to join behind the dorsal tubercle and are then continued backwards as a single fold of ciliated epithelium, the dorsal lamina (see fig. 3).

The free edge of this peripharyngeal band is slightly swollen and is covered by a tract of ciliated columnar cells (see figs. 6 and 7). Immediately anterior to this band, there is a slight groove, the "gouttière péricoronale" of French zoologists, and, in some cases, its anterior lip is raised to form a peripharyngeal membrane (see fig. 7), which is covered by non-ciliated squamous epithelium, similar to that which lines the whole of the prebranchial zone. (In this case, this anterior peripharyngeal band is in the condition described by Maurice in *Fragaroides* and by Oka in *Cyathocormus*.) No case has been found showing two *ciliated* peripharyngeal bands, such as are characteristic of most Simple Ascidians, but the two extremes for this genus (*Botryllus*)—a single ciliated peripharyngeal band, corresponding to the posterior band of other Ascidians, and two peripharyngeal bands, the posterior covered with ciliated cubical epithelium, the anterior with non-ciliated squamous epithelium, I have found represented by two adjacent ascidiozooids belonging to the same system and of like age! The anterior band, when present, is a continuous closed ring completely surrounding the pharynx. It passes across in front of the anterior end of the endostyle and, in the mid-dorsal region, becomes indistinct and merges with the raised dorsal tubercle. The whole of this region is covered by cubical ciliated epithelium.

[In *Styelopsis grossularia*, van Beneden, which probably comes as near *Botryllus* phylogenetically as any of the Simple Ascidians, the posterior band, alone, has the typical ciliated epithelium. The anterior band is a narrow fold of membrane covered by undifferentiated epithelium.]

DORSAL LAMINA.

Where the two ends of the posterior peripharyngeal band meet one another, they are raised to form the dorsal lamina, whose free edge is bent over to the right (see fig. 11). The epithelial cells of the tract lying immediately to the right of this fold are columnar and bear strong cilia; the lamina itself is covered, on its concave side and to within a short distance of the base on its convex side, with cubical ciliated epithelium. Between these two layers of cells is a narrow blood space. This dorsal lamina extends from just behind the dorsal tubercle anteriorly to the oesophageal opening posteriorly, along the mid-dorsal line of the branchial sac. It terminates on the left lip of the oesophageal opening.

[This lamina is essentially similar in origin and function to the dorsal languets of some Simple Ascidians such as *Ciona*.]

THE BRANCHIAL SAC AS AN ORGAN OF NUTRITION.

Sessile animals, such as the majority of the Tunicata, must have some special mechanism for bringing the food within reach of the gut, if they are to be nourished at all and, in the Ascidians, the branchial sac supplies this need. The ciliary and mucus-secreting action of the endostyle, peripharyngeal bands and dorsal lamina has long been known, but the way in which they work together has, until recently, been somewhat misinterpreted. It was formerly supposed that the cilia bordering the stigmata kept up a constant flow of water in through the branchial aperture and out to the atrium and thence to the exterior, but that they were powerless to sift out the minute planktonic organisms on which the animal feeds. In order to entrap these, the glandular tracts of the endostyle secreted a cord of mucus, which was passed forwards by the cilia of the groove. This cord was supposed to divide into two parts at the anterior end, and these passed round the prebranchial zone of the pharynx between the peripharyngeal bands. Here, at the entrance of the alimentary canal, the food particles in the incoming stream were wafted by the cilia of the peripharyngeal bands against the mucus cord, in which they were embedded. On reaching the mid-dorsal region, the two cords, with their entangled food particles, were reunited and passed backwards by the dorsal lamina (or, in some forms, the dorsal languets) to the mouth of the oesophagus, where mucus and food were together ingested. So far as it goes, this description is partly correct, but only a small fraction of the food is caught in this manner. In 1913, Orton made further observations and compared the results with the methods of food capture employed by Amphioxus and certain Lamellibranchs. His results are convincing, and may easily be confirmed by sprinkling some harmless colouring matter, such as powdered carmine, in the water over some actively feeding Ascidians. The first and most striking fact to be noticed is that the in-flowing current is so strong that most of the coloured particles are shot past the tentacles and peripharyngeal bands and that they fall out of the current and settle against the pharyngeal walls only after they are well within the branchial sac. The second fact is that those particles which fall onto the endostyle, instead of being entangled in mucus and carried forward, are immediately shot off again to one side or the other so as to fall against the stigmata. Next, it will be noticed that (at any rate in forms such as the Botryllidae) the cilia on the gill bars are of such a length that they practically meet across the stigmata (see fig. 8), so that it would be extremely difficult for any particles to escape along with the outgoing water. Partly by ciliary action and partly, according to Orton, by the waving to and fro of the longitudinal bars, the food is rolled in mucus secreted by the endostyle and thrown out over the walls of the branchial sac, and is then passed towards the mid-dorsal line. The cilia on the outer convex side of the dorsal lamina beat towards its free edge and those on the inner side beat away from its free edge, so that the particles will eventually be collected into the hollow, where they will be entangled by mucus and carried into the oesophagus, as described by the earlier writers.

ALIMENTARY CANAL (fig. 29).

The pharynx which, in Ascidians, is so highly modified as an organ of respiration and nutrition, has already been described. It remains to consider the rest of the alimentary canal.

At the posterior end of the branchial sac, a ciliated opening leads into the oesophagus, a short curved tube, whose walls consist of a single layer of ciliated columnar epithelium (see fig. 24). After running ventrally for a short distance, the canal expands to form the stomach, whose walls are folded longitudinally forming from six to eight shallow diverticula extending along its whole length. At the base of each of these grooves runs a tract of cells bearing very long cilia, while the cells on the ridges between the grooves bear short close cilia, similar to those found along the oesophageal walls. Between these bands of ciliated cells, on the sides of the furrows, are non-ciliated glandular cells which contain, during life, yellowish brown globules. In a few cases, however, sections show that the sides of these grooves are also thickly fringed with short close cilia (cf. figs. 30, 31 and 32).

The intestine curves forward, to the left and dorsally; it then turns posteriorly and the anus is situated close to the oesophagus, but opens into the atrium. The gut is ciliated throughout on the inside. The walls of the anterior loop of the intestine are formed of cubical epithelium and contain numerous pigmented globules of a yellowish brown colour, which appear to be identical with those in the stomach wall (see fig. 23). Further back the epithelium is flatter but still ciliated (see fig. 26), while the rectum is lined by columnar cells, each of which bears a single strong flagellum (see fig. 25).

Pyloric caecum. A diverticulum is given off from the left side of the stomach (see fig. 29). It curves anteriorly and ends blindly before reaching the intestine. Its walls consist of columnar ciliated cells and there are apparently some glandular cells towards the extremity (see fig. 27). During life these are swollen with a clear greenish fluid.

Refringent organ. The wall of the intestine behind the pigmented area is covered by a system of finely branched tubules, ending in numerous globular swellings, which lie close against the intestinal wall (see figs. 26 and 29). These tubules are formed by the dichotomous branching of a single duct, which enters the pyloric caecum close to the stomach-wall (see figs. 21 and 22). A few (not more than six) of these tubules run along the wall of the intestine towards the anus and end blindly with no terminal swelling (see fig. 29). One or two also run forward onto the curved portion of the intestine. According to Della Valle, Chandelon and Colton, the flattened epithelial cells of which this organ consists bear flagella which are long in the ampullae and short in the ducts (see fig. 28). I have been unable to find any trace of these in *Botryllus* in either living or preserved material, although in living specimens of *Botrylloides* the refringent organ and alimentary canal are so transparent that the flagella of the intestinal wall are clearly visible *through* the ampullae. (Cilia have also been recorded in this organ in *Perophora*, *Doliolum* and *Microcosmus*.) Both tubules and ampullae are perfectly colourless and highly refractile in the living state. For this reason, the name " refringent organ " seems the most appropriate of those which have been given to this structure by its various investigators.*

Various suggestions have been made as to the function of this organ. It has been considered as a true liver (Hancock), a digestive gland (Chandelon, Della Valle, and others), a part of the blood vascular system (Kupffer and Roule), a kidney of accumulation (Roule), an absorptive organ (Lister, Huxley, Pizon and Lefevre), and as an organ of nitrogenous excretion (Kowalewski and Colton).

The position of the organ and its duct, besides certain experiments by Henri, showing the presence of a substance similar to amylase, supports the view that the refringent organ is a digestive gland. Kupffer and Roule's opinion is based on injections, which must always be rather doubtful in an organism with tissues as delicate as those of an Ascidian. The fact that many investigators have described in detail cilia or flagella directed towards the opening of the duct is unfavourable to the idea of an absorptive function, while the suggestion that it is an excretory organ, although in many ways attractive—especially since no other organ of excretion has been found—has not yet been definitely proved.

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* The name " Pyloric gland " is also in general use, but, while the function remains doubtful, it seems important that the name should be absolutely non-committal.

DORSAL TUBERCLE.

In the prebranchial zone of the pharynx, in the mid-dorsal line, is situated an elliptical papilla known as the dorsal tubercle. It lies in a bay formed where the two ends of the peripharyngeal band meet and become the dorsal lamina (see fig. 13); its surface is covered by cubical ciliated cells and, on the summit, a small circular opening leads into a wide ciliated funnel occupying most of the cavity of the tubercle (see fig. 15). Into this funnel opens the duct of the neural gland.

NEURAL GLAND.

This gland, in the whole group of the Botryllidae, is situated *dorsally* to the nerve ganglion, a character shared only with the Cynthiidae among the Simple Ascidians (see figs. 13, 14 and 15). Following the duct backwards from the dorsal tubercle, we find it enlarging to form a simple sac-like dilatation, which is usually more or less filled with loose disintegrating cells, forming a spongy mass. This lies over the anterior part of the ganglion. The walls then close in again and often the lumen is completely obliterated, so that only a narrow strand of cells passes over the posterior end of the ganglion. Here the gland once more enlarges to form a slight swelling, lying a little to the right of the middle line. From this the organ, now a solid mass of cells, turns sharply downwards and forwards and merges imperceptibly with the cells of the posterior end of the ganglion.

FUNCTION OF THE NEURAL GLAND AND DORSAL TUBERCLE.

Many of the earlier investigators of Ascidians described the dorsal tubercle and ciliated funnel as an olfactory organ. Julin put forward the view, which has been widely accepted among zoologists, that the neural gland is the morphological equivalent of the hypophysis cerebri in vertebrates and he also suggested an excretory function. In his view the dorsal tubercle would be merely the opening of the duct from the gland.

Up to the present time, no definite decision has been reached as to the function of the neural gland, but the fact that Metcalf and Hunter have shown that the ciliated funnel is richly innervated seems to re-establish the dorsal tubercle as a sense organ of some kind. Probably, as suggested by Herdman, the connection between the two structures (the duct of a gland and a prepharyngeal sense organ) is a secondary and purely accidental relationship.

NERVE GANGLION AND CHIEF NERVES (fig. 13).

The ganglion itself—all that remains in the adult Ascidian to represent the central nervous system of a chordate animal—lies in the mid-dorsal line of the body-wall immediately anterior to the first row of stigmata. It is oval in shape and consists of a closely packed mass of nerve fibrils—the nuclei of the nerve cells being arranged round the periphery.

Two pairs of nerves are easily distinguishable arising from the ganglion—an anterior pair, running around the pharynx immediately posterior to the peripharyngeal band, each of which gives off a branch in the direction of the branchial aperture, and a posterior pair, which break up soon after their origin into branches, which are difficult to trace. It is also possible to make out two very fine nerves leaving the anterior end of the ganglion near the middle line and another pair of very fine lateral nerves.

Pizon describes other nerves, which he has traced for considerable distances, but these are so fine and indistinct that it is almost impossible to be certain of their course.

HEART, BLOOD, ETC.

The blood circulatory system of *Botryllus* is essentially similar to that of the Simple Ascidians, the chief modification being the enormous development of the test vessels to form the colonial vascular system.

The heart is situated in the mesoderm in the postero-ventral region, slightly to the right of the middle line; it is almost immediately below the posterior fold. It consists of a delicate swollen tube formed as an invagination in the wall of the pericardium (see figs. 18, 19 and 20). It contracts rhythmically, forcing the blood stream through the vessels and sinuses of the body and out through the test to the terminal knobs at the surface of the colony. From time to time, on the average about once in every two minutes in the adult zooid, the direction of the flow changes. The heart's pulsations become slower and apparently more difficult, then they cease altogether and the blood cells lie motionless or oscillate feebly for a few seconds. Suddenly the flow is reversed and the blood pours back through the heart to be forced out in the opposite direction. This alternation in the direction of the heart beats may be due merely, as Pizon* suggests, to the alternating pressure in the sinuses of the body and in the vessels of the test. This explanation for the extraordinary alternation, in the Tunicata, between a systemic and a respiratory type of circulation does seem the most reasonable that has yet been advanced and it is especially strengthened by the fact that, in the

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* Hancock, Linn. Soc. Journ. Zoology, Vol. IX, evidently had the same idea although he does not express it so definitely.

Botryllidae, as Pizon has shown, the direction of flow changes in the ampullae and test vessels *before* the heart reverses. He also describes how this reversal takes place simultaneously in every ascidiozooid throughout the colony, and this is, in the main, true, though the synchronisation is not always so accurate as in his records. Thus, in my observations, the direction of flow may be seen to change in one ascidiozooid from five to ten seconds later than in an adjacent one, and even greater irregularities have been recorded.

The mantle is penetrated in all directions by blood sinuses, but the more important of these may easily be traced in the living organism, by following the blood corpuscles as they circulate around the body. From the anterior extremity of the heart, a large vessel runs forward below the endostyle, sending off branches to the right and left which pass into the transverse bars of the branchial sac, and supply the circulation around the stigmata. This great ventral vessel gives off the " subendostylar test vessel," which passes out into the test and to the periphery of the colony, where it ultimately anastomoses with a large peripheral vessel. This completely surrounds the colony and is richly supplied with terminal knobs. At the anterior extremity of the endostyle, the ventral vessel divides into two parts which run around the pharynx close beneath the peripharyngeal band. They join again on the dorsal surface and pass back as a single dorsal vessel, lying just over the dorsal lamina. A second vessel is given off to the test from each ascidiozooid. This is the " subintestinal vessel " and arises opposite the anterior loop of the intestine. It also ultimately makes its way to the peripheral vessel. Each blastozooid also retains the vascular peduncle, by which it was originally connected with the parent zooid. After the latter has died and disintegrated, the peduncles of the two buds become united, the position of the former zooid being marked merely by a slight swelling at the junction of the five vessels (see Text-fig. 1).

SPECIAL FUNCTIONS OF THE COLONIAL VASCULAR SYSTEM.

The colonial vascular system in *Botryllus* has several rather special functions. Besides distributing nutriment in the ordinary way throughout the colony, the test vessels remove the products of disintegration from each generation in turn and redistribute them to the developing buds. The heart is the last organ to degenerate and continues to beat for several days after all other structures have disappeared, driving the cells in loose bundles out along the test vessels and into the next generation of buds, which are then just at the stage of their most rapid growth. Then the terminal knobs of the test vessels play an important part in the secretion of the tunicine of which the test is composed, a function which they possess in common with the ectoderm all over the body. Their third function, that of respiration, was first suggested by Herdman, who showed how the blood was brought to the surface in the terminal knobs and there aerated, the test vessels thus forming a subsidiary respiratory organ, especially well developed in those Tunicates which have the least efficient branchial sacs.

GONADS (see figs. 33, 34 and 35).

The gonad is paired and is placed laterally in the body-wall. Its products—ripe spermatozoa or advanced embryos—are shed into the peribranchial cavity.

Botryllus is hermaphrodite, but true self-fertilisation cannot take place. It is sometimes described as protogynous because, in any one blastozooid, the gonad in the early stage shows numerous ova, while later there are no signs of these, ripe spermatozoa alone being present. Actually the case is much more unusual and more interesting than any ordinary case of protogyny for, in the oozooid, the gonads appear as small masses of undifferentiated cells, a few of which are soon distinguishable as young ova. None of these, however, reach maturity *in situ*, but *migrate* in the blood stream into the first blastozooid, where they again form laterally placed gonads. They continue to develop, although they still do not reach maturity, but migrate again into the next generation of blastozooids. It is not until the fifth generation (the oozooid being the first) that mature sexual products are found, and ova are not fertilised until the seventh or eighth generation. That is to say, the young ova visible in the oozooid migrate through six subsequent generations of blastozooids before they are ready to be fertilised. Throughout the whole life-history of the colony, the same process takes place. Although each generation adds to the germ cells, those ova which are mature were derived from the mesoderm of a zooid about four generations back.

The first generations to become sexually mature, therefore, are apparently male, for the ova have already migrated into the buds. The spermatozoa are liberated into the peribranchial cavity, but probably most of them are wasted since the whole colony will, at that time, contain no ripe ova. In older colonies, where a few ova ripen in each blastozooid, these may be fertilised by spermatozoa from the same zooid, but this is not simple self-fertilisation, since the ova are several (asexual) generations older than the spermatozoa.*

* As Pizon showed, it is impossible for one generation to fertilise another because, at the time when the spermatozoa are liberated, the preceding generation is undergoing degeneration, while the following generation is still completely enclosed within the common test. It is quite possible, however, for separate ascidiozooids of like age from the same colony or from neighbouring colonies to fertilise one another since the peribranchial cavity, into which the spermatozoa are shed, communicates freely with the exterior.

In a young bud, then, in any advanced colony, the gonads have the following appearance (see fig. 33). About half a dozen immature, but very clearly differentiated ova are present in the dorsal region on each side. These are already partly or wholly surrounded by follicle cells. Other smaller, but already distinct, ova are also present along with a considerable number of undifferentiated cells. In the more ventral part of the gland there is another mass of small cells whose outlines are not clearly defined. The nuclei, however, are very distinct and these are destined to form the male part of the gonad.

In the sexually mature zooid only one or two ova remain, and these will develop into embryos within the body of the parent, and will finally escape by rupturing the epithelium lining the peribranchial cavity. The male part of the gland is now well developed and is a lobed organ consisting of a peripheral layer of epithelial cells and an inner mass of spermatozoa (see fig. 35). The investing epithelium is continued as a short duct opening into the peribranchial cavity. Through this the ripe spermatozoa escape.

EMBRYOLOGY AND DEVELOPMENT.*

The embryology of the Compound Ascidians is essentially similar to that of Simple Ascidians, although it is to some extent condensed and certain stages are telescoped out of existence.

Embryonic Envelopes.

The mature ovum in *Botryllus* is surrounded by two concentric follicles.

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* This outline follows the results of Pizon's work. The matter is at present under reinvestigation by Professor and Miss Garstang and their results so far do not wholly agree with those of Pizon. It is probable, therefore, that our ideas on the development of the Botryllidae may shortly have to be revised.

The external follicle consists of cubical cells and this envelope is left behind when the larva falls into the peribranchial cavity.

The internal follicle consists of a single layer of flattened cells. It is produced by the proliferation of the external follicle and forms the outer envelope of the larva after this is set free.

Inside the internal follicle and in direct contact with the egg is a more or less complete layer of spherical or slightly flattened cells, which are often known as "testa cells," but have no connection with the test or tunic of the adult Ascidian.

Various opinions have been held as to the origin of these envelopes and the function of the testa cells, and it is possible that they do not arise in the same manner in all Ascidians. In *Botryllus*, it seems that they are all three derived from ovarian cells, which have never differentiated to form ova, but the function of the testa cells is more uncertain. According to Pizon and others they are formed within the egg itself and are thrown out as waste material. Certainly they degenerate early in development.

Before fertilisation takes place, the external follicle, which lies against the peribranchial membrane, evaginates and forms a short duct opening into the peribranchial cavity. By this the spermatozoa, which are free in the atrium, gain access to the ovum ; the duct does not remain as an outlet for the embryo but closes once the egg is fertilised. During the subsequent development, the embryo hangs into the peribranchial cavity suspended by the surrounding peribranchial membrane, which it has pushed before it.

Segmentation results in the formation, first, of a blastula and then of a gastrula, the processes being closely parallel to those in the embryology of Amphioxus. The rounded head end becomes clearly distinguishable from the more elongated tail region, in which the notochord is laid down as a single layer of large cylindrical cells derived from the dorsal hypoblast. Around this tail region are grouped the testa cells. The central nervous system is formed by the invagination of the epiblast along the mid-dorsal line giving rise to a furrow, which closes in from behind forwards, leaving the nerve tube in connection with the archenteron for a time by the neurenteric canal.

The archenteron next begins to bud off secondary cavities and about the same time the sense organs are differentiated at the anterior end of the nerve tube.

At this stage, the lining membrane of the parental atrium and the external follicle of the embryo burst, so that the embryo, still enclosed in the internal follicle, falls into the peribranchial cavity. The cellulose test, which first appears soon after the formation of the ectoderm, now thickens, pushing the testa cells before it, so that these come to lie close against the follicle. The cells of this inner follicle become more and more attenuated and finally, when the larva is fixed and beginning to bud, they disappear altogether. Fixation is effected by an ectodermal outgrowth from the anterior region of the embryo, and this is surrounded (in the free-swimming stage) by eight ectodermal ampullae, which are destined to give rise to the colonial vascular system (see fig. 36). The central lobe carries three fixation papillae consisting of glandular cells. These send out threads which secrete a gelatinous substance, and this serves to attach the larva to the substratum. In the free-swimming larva, the nervous system consists of an anterior vesicle containing the sense organs, a visceral cord running dorsally above the intestine, while in the caudal region the lumen of the neural tube still persists.

The various cavities of the larva are formed as follows :—

At an early stage, while the embryo (a simple gastrula) is still enclosed by the embryonic membranes and the lining of the maternal atrium, the archenteron gives off four diverticula.

1. A dorsal outgrowth extending from behind forwards between the ectoderm and the nerve tube and ending blindly near the anterior end of the larva. This will give rise to the neural gland and its ciliated duct.
2. A ventral diverticulum—the pericardium.
3. Two lateral symmetrical outgrowths which will give rise to the peribranchial cavity.

The first of these diverticula grows forward and opens again at its anterior end close to the future position of the branchial aperture. The posterior opening of the tube then closes and the neural gland is formed by proliferation of the cells of its wall. There is no connection during development between the ganglion and the neural gland.

The pericardial vesicle becomes separated from the primitive endodermal vesicle and lies ventrally beneath the pharynx. Later a furrow appears in its dorsal wall. This invagination ultimately closes off in the middle, leaving a double-walled tube open at both ends—the heart, surrounded by the pericardium (see figs. 18, 19 and 20).

The two symmetrical lateral outgrowths extend ventrally round the pharynx but remain connected with each other dorsally even after they have become completely separated from the alimentary canal. Posteriorly these two peribranchial cavities each give off an outgrowth. These lie at the sides of the gut and together form the perivisceral space.

The remainder of the primitive endodermal vesicle forms the branchial sac and alimentary canal. The pharyngeal region is much enlarged and its cavity becomes connected with the surrounding peribranchial space by the formation of gill slits or primary stigmata. Thickenings appear opposite one another in the epithelium lining the pharynx and the atrial cavity. These come in contact, fuse together and finally openings are formed in them. Thus, in the early fixed zooid, four pairs of transversely elongated primary stigmata are found. Each of these extends right across the side of the pharynx from the dorsal region to the endostyle. Later they divide, so that a transverse *row* of small secondary stigmata is formed. At a still later stage five rows are visible on each side, but probably there are never more than this in the oozooid.

The posterior part of the mesenteron elongates and becomes twisted on itself to form the gut, its posterior end opening into the atrial cavity. Two outgrowths from it form the pyloric caecum and the refringent organ.

The branchial and atrial apertures are formed partly by invagination from the exterior and partly by evagination of the lining of the branchial sac and atrial cavity respectively. They are situated at the anterior and posterior ends of the dorsal surface.

Organogenesis in the bud follows a course parallel on the whole with that of the larva, though the nervous system and the stigmata are exceptions to this rule.

The origin of the nerve ganglion in the blastozooid is somewhat uncertain, but it certainly does not arise as a dorsal ectodermal tube. Possibly, as Pizon states, it is derived directly from nervous elements in the preceding asexual generation.

The stigmata of the bud are formed directly as transverse rows of small openings and are not secondarily produced by the division of primary transversely elongated gill slits.

SPECIES OF *BOTRYLLUS*.

Many species and varieties of *Botryllus* have been described, especially by Giard, but recently several workers, notably Bancroft, have come to the conclusion that the colour characters which have been used to distinguish the different "species" are inconstant and that no morphological differences occur. It seems best, therefore, to conclude that only one true species of *Botryllus* has yet been described, viz., *Botryllus schlosseri* (Pallas) Savigny—a species which varies widely in its range of colours. There do, however, seem to be several very distinct colour varieties, formerly described as species, such as *B. smaragdus* and *B. violaceus*, but even these merge imperceptibly into one another. Colonies from sister oozooids may show widely different colouration, while the same colony at different stages in its development and under different external conditions may also change greatly.

COLOURATION.

The various colour varieties just mentioned owe their characteristics to pigment granules carried in the blood either free or enclosed in pigment cells or chromocytes (see fig. 8). Pizon first drew attention to the motility of these granules within their cells—a common feature throughout the whole animal kingdom—and he also traced their origin in each blastozooid from the degenerated cells of the parent. Even the embryo receives a certain number of pigment granules from the maternal blood and these invade the previously colourless blood cells and the differentiated tissues. Each subsequent generation of blastozooids becomes more and more strongly pigmented owing to the further disintegration of cellular elements.

Generally each colony has pigment of three colours—pale opaque white or yellowish green cells, dark blue, brown or black cells and red or orange cells. The colour differences in colonies are due to differences in the relative numbers and the distribution of these pigment cells. Most of the patterns represent structural features in the ascidiozooid—for instance, the positions of the chief blood sinuses are often distinguishable and the endostyle is especially deeply pigmented. Parts of the alimentary canal are also strongly coloured but this is chiefly due to the yellowish brown contents of the glandular cells. The branchial sac is often pigmented along the transverse and internal longitudinal bars only (see fig. 8). This is apparently due to the fact that the pigment cells, which are nearly all of large size, are unable to pass into the narrower longitudinal interstigmatic vessels.

SYSTEMATIC POSITION OF THE BOTRYLLIDAE.

On the strength of their power of budding to form colonies of ascidiozooids intimately connected with each other and enveloped in a common test, the Botryllidae are included in the sub-order Ascidiae Compositae. This is a convenient but unnatural division, for the details of morphology in the different families indicate that the colonial habit has appeared more than once in the evolution of the Ascidiacea. We have seen that *Botryllus* has simple tentacles, the alimentary canal in the form of a loop on the left side of the branchial sac, non-papillated internal longitudinal bars and the gonads placed laterally on the inner surface of the mantle. In these points it resembles the Styelinae, a sub-family of the Simple Ascidians, but differs from most of the other Compound Ascidians. Probably, then, the ancestral Botryllidae diverged from the main stem of the Ascidiae

Simplices close to the point of origin of the Styelinae. Another family of the Compound Ascidians, the Polystyelidae, differs from the Styelinae in nothing but the power of budding—even common cloacal cavities are absent—and it is possible that the Botryllidae may have been evolved from the Polystyelidae by the arrangement of the ascidiozooids into systems.

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EXPLANATION OF PLATES

REFERENCE LETTERS

a. = Anus.
at. = Atrium.
at. ap. = Atrial aperture.
at. l. = Atrial lobe.
b. = Bud.
b. c. = Bladder cell.
br. ap. = Branchial aperture.
br. s. = Branchial sac.
c. c. = Common cloaca.
c. f. = Ciliated funnel.
d. l. = Dorsal lamina.
d. r. o. = Duct of refringent organ.
d. t. = Dorsal tubercle.
end. = Endostyle.
e. p. = Ectodermal processes.
g. = Ganglion.
h. = Heart.
i. l. b. = Internal longitudinal bar.
int. = Intestine.
i. s. v. = Interstigmatic vessel
l. b. = Longitudinal bar.
l. t. = Larval tail.
n. g. = Neural gland.
oes. = Oesophageal opening.
p. b. = Peribranchial space.
p. c. = Pigment cell.
per. = Pericardium.
p. f. = Posterior fold.
p. p. b. = Peripharyngeal band.
p. p. m. = Peripharyngeal membrane.
pyl. = Pyloric caecum.
r. o. = Refringent organ.
s. o. = Larval sense organ.
st. = Stomach.
stig. = Stigma.
t. = Test.
t. b. = Transverse bar.
ten. = Tentacle.
t. f. = Test fibrils.
t. k. = Terminal knob.
t. v. = Test vessel.
♂ = Male gonad.
♂ *d.* = Duct of male gonad.
♀ = Ovum.

PLATE I.

Fig. 1. Corm of *Botryllus* consisting of several systems. In the middle of the figure an ascidiozooid is being pushed out of an already crowded system. × 18.

PLATE II.

2. Diagram showing a single ascidiozooid embedded in the common test. The animal has been bisected by a median longitudinal incision, and the right half removed. Thus the left side of the branchial sac is seen from the interior. × 40.

Fig. 3. Diagram showing a single ascidiozooid from the dorsal surface. × 40.

Fig. 4. Diagram showing a single ascidiozooid from the ventral surface. × 40.

PLATE III.

Fig. 5. Tentacles as seen by looking in at the branchial aperture of a living ascidiozooid. × 80.

Fig. 6. Transverse section of the peripharyngeal band. × 200.

Fig. 7. Transverse section of the peripharyngeal band, peripharyngeal membrane and one tentacle. × 200.

Fig. 8. Part of the branchial sac, showing stigmata and one of the internal longitudinal bars. × 400.

Fig. 9. Anterior extremity of the endostyle, showing that the peripharyngeal band is continuous with the lips of the endostyle. × 200.

Fig. 10. Transverse section of part of the branchial sac, showing two internal longitudinal bars. × 400.

Fig. 11. Transverse section of the dorsal lamina. × 400.

Fig. 12. Transverse section of the endostyle. × 400.

PLATE IV.

Fig. 13. General view, from the dorsal surface, of the neural gland and ganglion, showing the distribution of the chief nerves. × 80.

Figs. 14 and 15. Diagrams showing the inter-relationship of the ganglion and neural gland. × 180. Fig. 14, from the dorsal surface. Fig. 15, median longitudinal section. [Partly after Metcalf.]

Fig. 16. Section through the test showing two vessels. × 400.

Fig. 17. General view of a lobe of test with vessels. × 30.

Figs. 18, 19 and 20. Three transverse sections of part of a young ascidiozooid, showing how the heart is formed by an invagination in the wall of the pericardium. × 400.

Fig. 18. Section through the posterior end of the heart.

Fig. 19. Section through the middle of the heart.

Fig. 20. Section through the anterior end of the heart.

PLATE V.

Figs. 21 and 22. Sections showing the origin and dichotomous branching of the duct of the refringent organ. × 100.

Fig. 23. A few cells from the wall of the anterior loop of the intestine. × 400.

Fig. 24. A few cells from the wall of the oesophagus. × 400.

Fig. 25. A few cells from the wall of the rectum. × 400.

Fig. 26. Longitudinal section through the intestine, showing the refringent organ. × 400.

Fig. 27. Transverse section of the pyloric caecum. × 400.

Fig. 28. Two ampullae of the pyloric gland. × 500. [After Colton.]

Fig. 29. Alimentary canal from the left side. × 180.

PLATE VI.

Figs. 30 and 31. Transverse sections through the stomach. × 80. Fig. 30 shows thick fringes of cilia on the sides of the grooves. Fig. 31 shows no cilia but a cuticle on the sides of the grooves.

Fig. 32. Transverse section of a single groove in the stomach wall of *Polycyclus vallii*,* Lah., according to Lahille. × 180.

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* See footnote on p. 40.

Fig. 33. Transverse section of a bud, showing the rudiment of a secondary bud on each side. The male and female parts of the gonad can be distinguished. × 300.

Fig. 34. Transverse section of the body-wall of an older bud, showing the developing gonad. At this stage all the ova have migrated into the next generation, except those which are destined to become mature in this generation. In this case only one ovum remains. × 300.

Fig. 35. Transverse section of the body-wall of a mature ascidiozooid, showing the testis. × 300.

Fig. 36. Larva of *Polycyclus renieri.** × 40. [After Lahille.]

Fig. 37. Larva of *Sarcobotrylloides wyvillii.* × 80. [After Herdman.]

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* *Polycyclus* differs from *Botryllus* in nothing but the character of the corm which is thick and fleshy in *Polycyclus*, thin and encrusting in *Botryllus*. By many they are considered as a single genus. *Polycyclus* is found in deeper water than *Botryllus*.

Sarcobotrylloides bears the same relation to *Botrylloides* that *Polycyclus* bears to *Botryllus*.

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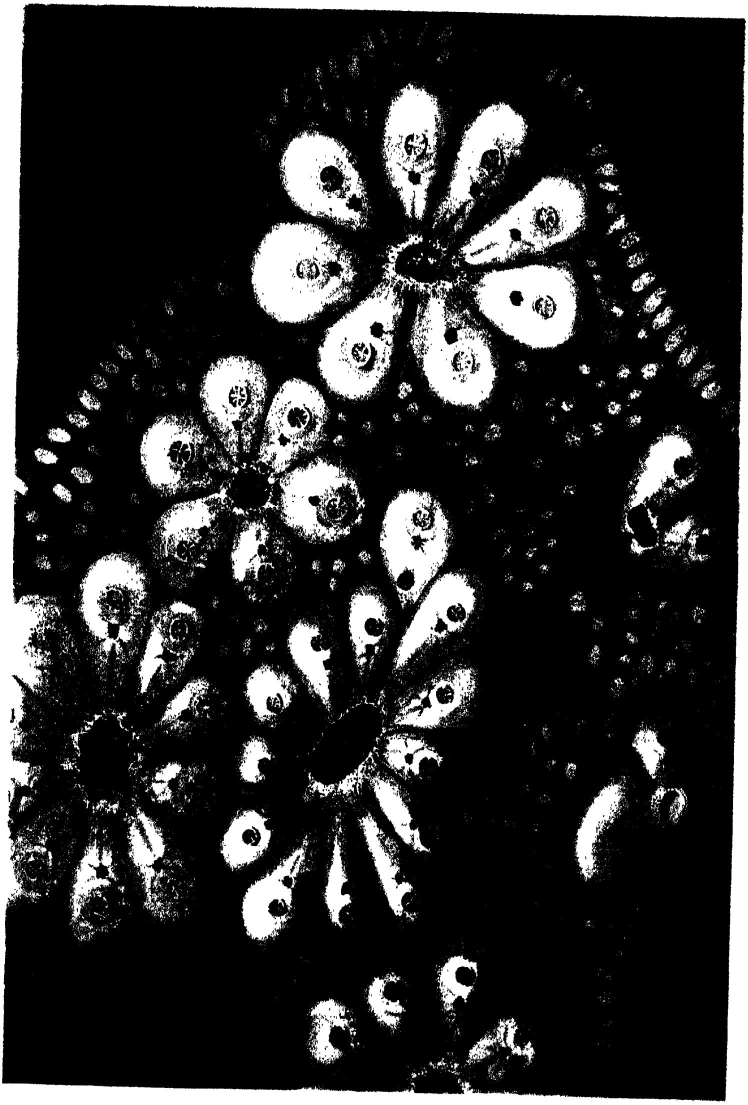

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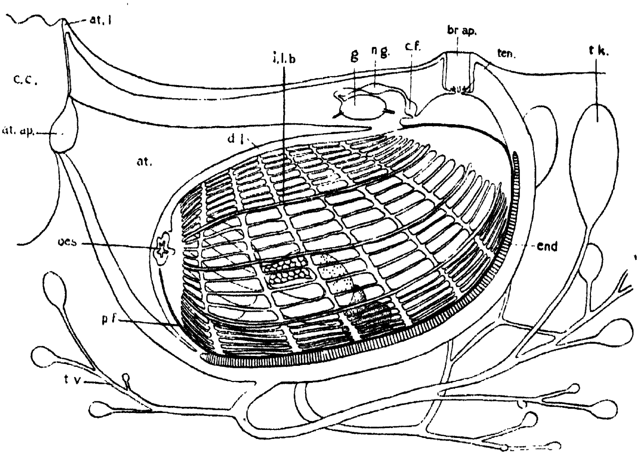
BOTRYLLUS



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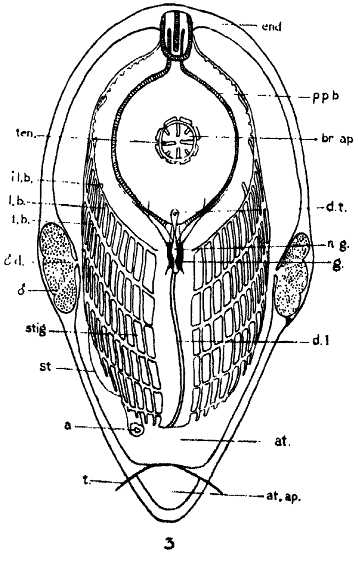

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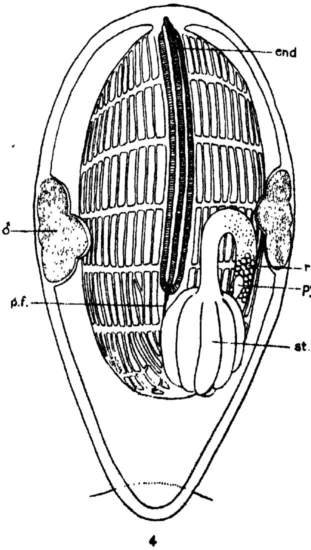

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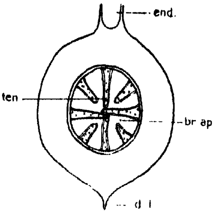
BOTRYLLUS

L.M.B.C. MEMOIR XXVI

PLATE III



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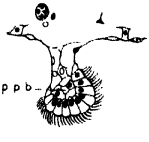

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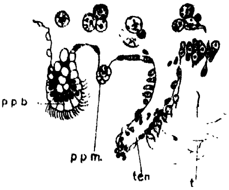

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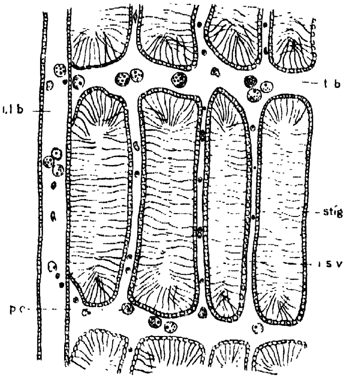

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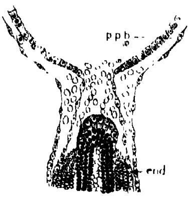

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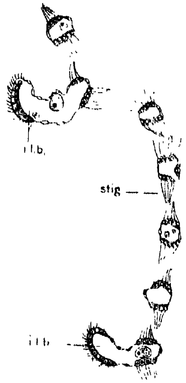

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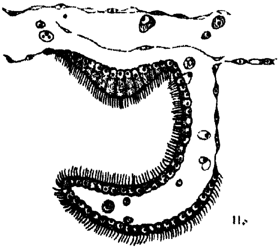

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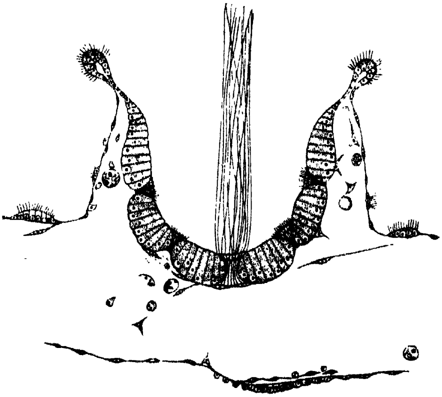

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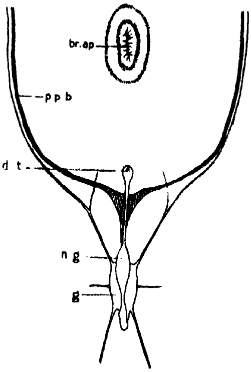

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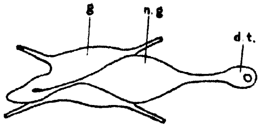

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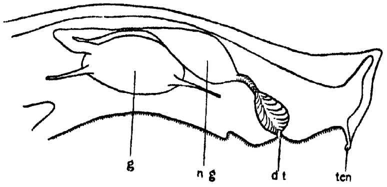

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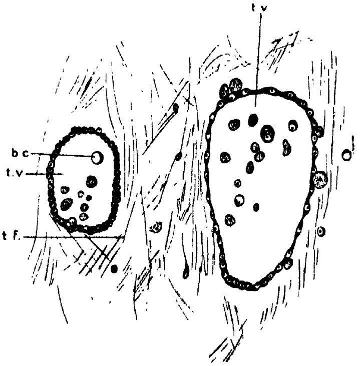

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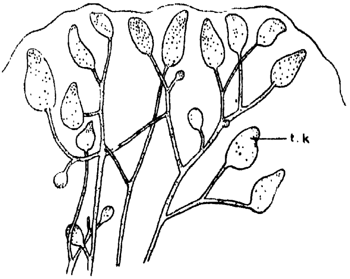

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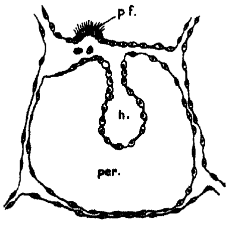

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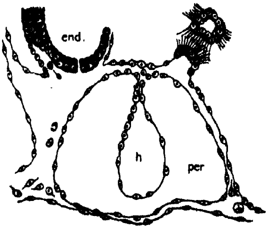

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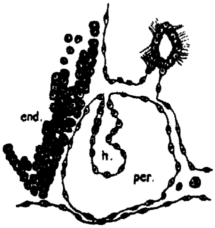

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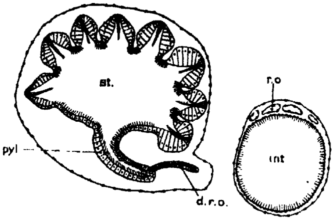
BOTRYLLUS



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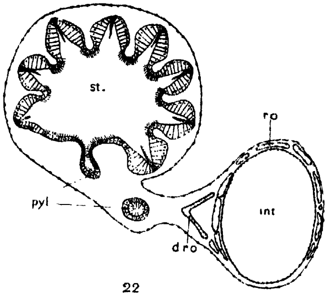

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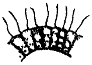

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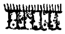

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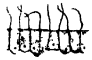

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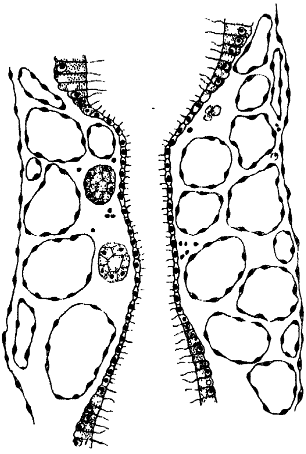

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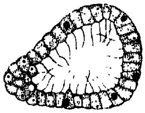

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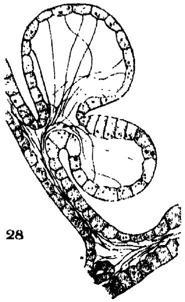

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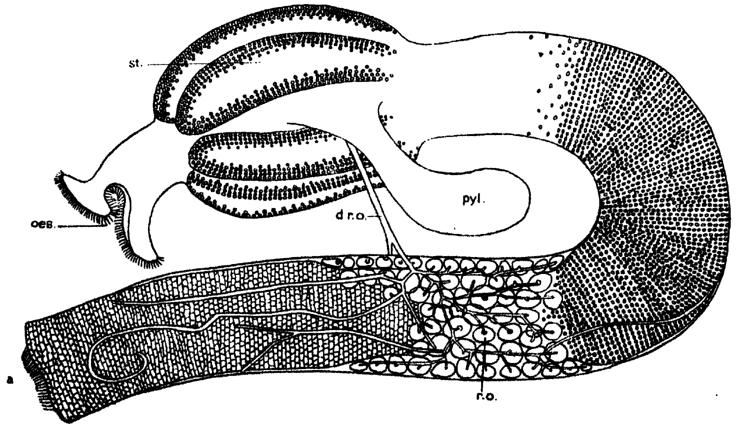

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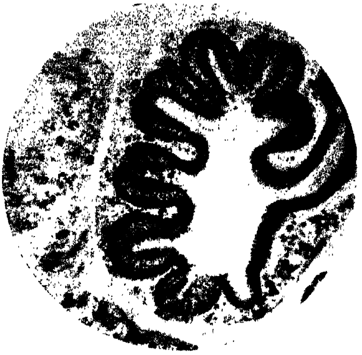
BOTRYLLUS



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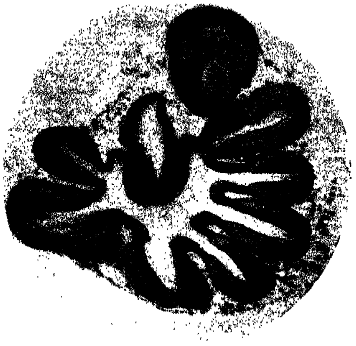

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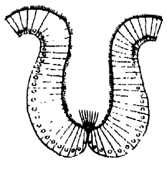

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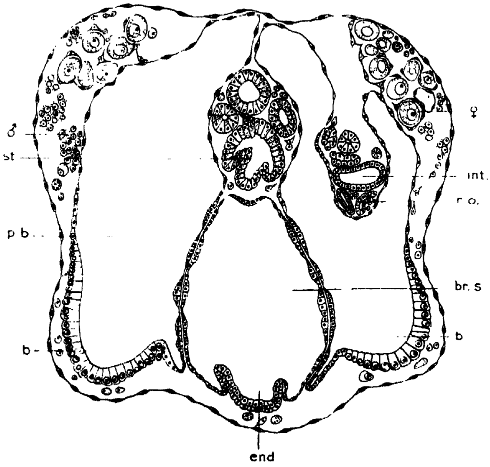

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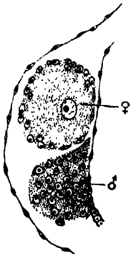

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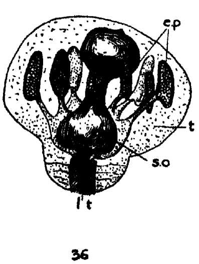

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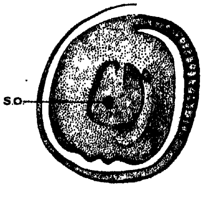

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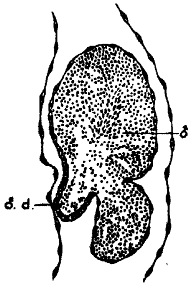

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